

Amendments to the Claims

This listing of claims will replace all prior versions and listings of all claims in the application.

Claims 1-27 (cancelled)

28. (Currently amended) A non-naturally occurring variant TNF- α protein comprising an amino acid sequence that has at least one amino acid substitution as compared to the wild-type human TNF- α sequence, wherein said variant TNF- α protein will interact with a naturally-occurring human TNF- α to form mixed trimers incapable of activation receptor signaling, wherein said substitutions are at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115, 140, and 143 ~~K112D, Y115T, D143K, D143K, D143R, and Y115I~~.

29 (New) An isolated TNF- α mixed trimer comprising:

a) at least a first variant monomer comprising one or more amino acid substitutions as compared to a naturally occurring human TNF- α monomer sequence comprising SEQ ID NO. 2; and

b) at least a second TNF- α monomer comprising a sequence different from said first monomer.

30. (New) An isolated TNF- α mixed trimer according to claim 29 wherein said second monomer comprises a naturally occurring human TNF- α monomer.

31. (New) An isolated TNF- α mixed trimer according to claim 29 in which two of the monomers comprise a naturally occurring human TNF- α monomer sequence.

32. (New) An isolated TNF- α mixed trimer according to claim 29 in which two of the monomers are variant monomers comprising one or more amino acid substitutions as compared to a naturally occurring human TNF- α monomer sequence.

33. (New) An isolated TNF- α mixed trimer according to claim 32 wherein said variant monomers have the same sequence.

34. (New) An isolated TNF- α mixed trimer according to claim 32 wherein said variant monomers have different sequences.

35. (New) An isolated TNF- α mixed trimer according to claim 29 wherein all three monomers are variant monomers.

36. (New) An isolated TNF- α mixed trimer according to any one of claims 29-35 in which the amino acid sequence comprising one or more amino acid substitutions as compared to a naturally occurring TNF- α monomer sequence comprises at least one amino acid substitution at a position selected from the group consisting of positions 21, 30, 31, 32, 33, 35, 65, 66, 67, 84, 111, 112, 115, 140, 143, 144, 145, 146, and 147.

37. (New) An isolated TNF- α mixed trimer according to claim 36, wherein at least one of said amino acid substitutions is selected from the group of substitutions consisting of D143E, D143N, D143S, A145R, A145K, A145E, E146K, E146R, and A84V.
38. (New) An isolated TNF- α mixed trimer according to claim 36, wherein at least one of said amino acid substitutions is selected from the group of substitutions consisting of K112D, Y115T, Y115I, D143K, and D143R.
39. (New) An isolated TNF- α mixed trimer according to claim 36, wherein said substitution comprises Y115T (SEQ ID NO.: 20).
40. (New) A glycosylated TNF- α trimer comprising three TNF- α monomers, at least one of which is a first variant monomer comprising one or more amino acid substitutions as compared to a naturally occurring human TNF- α monomer sequence comprising SEQ ID NO. 2.
41. (new) A glycosylated TNF- α trimer according to claim 40 wherein all three monomers are variant monomers.
42. (New) A glycosylated TNF- α trimer according to claim 41 wherein all three monomers have the same sequence.
43. (New) A method of forming mixed TNF- α trimers comprising combining:
- a) a first variant TNF- α trimer comprising at least one variant TNF- α comprising one or more amino acid substitutions as compared to a naturally occurring human TNF- α monomer sequence comprising SEQ ID NO. 2; and
 - b) a second TNF- α trimer comprising naturally occurring human TNF- α monomers;
- under conditions where mixed trimers are formed.
44. (New) A method according to claim 43 wherein said first variant trimer comprises three variant monomers.
45. (New) A method according to claim 44 wherein all monomers are glycosylated.
46. (New) A non-naturally occurring variant TNF- α protein comprising an amino acid sequence that has at least one amino acid substitution as compared to the wild-type human TNF- α sequence (SEQ ID NO: 2), wherein said substitutions are selected from the group consisting of Q21R, Q21K, N30E, R31V, R31L, N30D, R31I, R31D, R32D, R32E, R32S, R32H, R32T, A35T, A35S, G66Q, G66K, G66N, G66R, G66E, A111R, A111E, A111K, A111D, K112D, K112E, Y115Q, Y115K, Y115E, Y115N, Y115R, Y115F, Y115H, Y115M, Y115L, Y115I, Y115D, Y115T, Y115S, Y115V, D140R, D140K, D140Q, D140E, F144Q, F144H, F144N, E146N, E146K, E146D, E146K, E146Q, E146H, E146E, E146T and E146S.

47. (New) A non-naturally occurring variant TNF- α protein according to claim 46, wherein said substitutions are selected from the group consisting of Y115L, Y115V, Y115S, and E146Q.
48. (New) A non-naturally occurring variant TNF- α trimer according to claim 46-47, wherein said variant TNF- α trimer further comprises at least one covalent modification.
49. (New) A non-naturally occurring variant TNF- α trimer according to claim 48, wherein said covalent modification comprises a polyethylene glycol molecule.